Size Distribution and Fluorescent Characteristics of Colloidal CDOM: Indicators of the Provenance and Reactivity of CDOM in Coastal Waters?

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LONG-TERM GOAL

Our long-term research goal is to ascertain the nature and magnitude of optical effects (absorbance / fluorescence / scattering) in surface seawaters associated with the production and cycling of marine colloidal organic matter. We are particularly interested in determining how these effects are driven or modulated by the productivity dynamics of phytoplankton and marine heterotrophic bacteria in addition to terrestrially derived materials.

OBJECTIVES

Marine chromophoric dissolved organic matter (CDOM) imparts highly variable optical signatures in surface waters over short spatial and temporal scales for reasons not yet understood. While considerable research efforts are currently underway on the specific absorption and fluorescence characteristics of the bulk CDOM and FDOM, our primary objective is to follow the chromophoric signatures of different molecular weight fractions to determine if the production (or allochthonous input) and removal of colloidal organic matter contributes to the high variability in bulk CDOM. We know that a signficant fraction (10-40%) of non-living dissolved organic matter resides in the colloidal size fraction (1-1000 nm) (Benner et al., 1992; Buesseler et al., 1996; Chen and Schnitzer, 1989), and that this fraction is very reactive to both bio-degradation (to soluble substances) (Benner et al., 1992) and aggregation (to large sinking particles)(Baskaran et al., 1992; Chin et al., 1998; Moran and Buesseler, 1992) The challenge is to determine to what extent these dynamic, opposing processes influence the behavior of CDOM and FDOM in surface seawaters, and how these biogeochemical effects interweave with photochemical degradation pathways.

APPROACH

Our studies have focused on field measurements and laboratory incubation experiments aimed towards understanding how the optical characteristics of soluble and colloidal organic matter change under different phytoplankton growth conditions. Laboratory growth experiments are done using synthetic seawater to quantify that portion of CDOM and FDOM generated by phytoplankton during active (log phase) growth through extended senescent (stationary) phases. Results from these simplified systems are contrasted with CDOM collected from field stations in the Damariscotta River estuary and in other cruise-of-opportunity locations.
Size fractionation of CDOM and FDOM in our seawater samples is accomplished using Flow Field-Flow Fractionation (Flow FFF). Unlike conventional cross-flow filtration, which provides a single

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cutoff size, Flow FFF partitions organic phases into a size continuum, from soluble through to particulate (> 0.45 μm) sizes. Briefly, a flow field is applied at right angles to the channel flow within a shallow (~200 μm) ribbon-like chamber. Soluble fractions are driven through the membrane (1 KDa) on the accumulation wall, while colloidal components are driven to the accumulation wall. The resultant concentration gradient is opposed by diffusion (a function of colloidal size), resulting in colloids of different size being retained in different stream laminae. Unequal laminae velocities due to shear along the accumulation wall causes a well-defined separation of different colloidal size fractions according to the mean colloid proximity to the wall, which then is measured by UV absorption after the sample stream exits the flow chamber. A single wavelength detector (254 nm) has been primarily used for quantifying CDOM. In addition to yielding a soluble phase, the method provides a high resolution separation of organic matter into a continuous colloidal size spectrum.

Limitations of detection sensitivity have required that the entire colloid fraction be concentrated before analysis. We use on-line focusing for this purpose, whereby seawater (1-50 ml) is "focused" at the head of the channel before proceeding with the normal analysis mode. This approach has yielded sufficient sensitivity to measure the colloidal fraction.

Over the last year we have expanded the characterization of collodial CDOM, interfacing the channel outflow of FlowFFF to a 50 cm waveguide/CARY 50 spectrophotometer system. This improvement enables us to obtain multiple absorbance scans across the CDOM size continuum, that are then contrasted to the absorbance spectrum of the bulk dissolved sample. Subsequently, outflow from the waveguide is collected for analysis by excitation emission matrix spectroscopy (EEMS), with countour surface plots providing fingerprints of 3D fluorescence collected for different sample aliquots. EEMS has been shown to be an effective means for distinguishing between types of organic matter in seawater (Coble, 1996). In addition, absorbance scans were measured on each sample aliquot across the same excitation range to both characterize the absorbance characteristics and to enable the relative fluorescence efficiencies to be estimated. As a consequence, we are able to analyze the absorbance and fluorescence characteristics of different colloidal size fractions during a single sample run, and compare them with thecharateristics of the bulk dissolved (<0.4 μm) CDOM.

WORK COMPLETED

The early stages of this year's progress were directed at interfacing and testing the combined Flow-FFF, scanning spectrophotometer (waveguide) and scanning spectrofluometer system. This testing included both standard solutions and a limited suite of natural samples from the Damariscotta estuary. The focus flow rate was optimized and sample focus volume was increased to better accomodate the detection limits for absorbance and fluorescence determinations. The effect of increasing (50 ml) focus volume on peak retention times was determined for both standards and natural samples, and our findings showed that focusing the colloidal matter at the head of the Flow-FFF channel did not generate size artifacts due to aggregation of the colloidal phase.

Surface water samples have been, and continue to be, collected in the Damariscotta River estuary over winter, spring, summer and continuing through fall. The purpose here is to establish a baseline for how the combined effect of runoff, phytoplankton production, and macroalgal production influences the bulk and size distribution of CDOM and FDOM. This study has shown significant variations over time that appears to be related to the total chlorophyll concentrations in the estuary. In addition, monoclonal cultures of several different phytoplankton species have been reared in synthetic seawater and the colloidal CDOM and FDOM size spectrum measured (by Flow-FFF) at different growth stages

(early exponential, late exponential, senescence) to determine the size characteristics of optically active phytoplankton-derived materials.

Results from the laboratory cultures and seasonal field sampling in the Damariscotta River estuary are to be contrasted with a very recent cruise-of-opportunity study of the Juan de Fuca eddy region off Vancouver Island and the Washington State coastline. This latter shelf region provides an excellent comparison site to the confined, phytoplankton and macroalgal influenced Damariscotta estuary because it contains discrete surface waters influenced by upwelling, high in-situ primary production, general continential and offshore inputs. Flow-FFF analyses were conducted on board on >50 water samples collected from an underway pumping system using the optimized focusing method. Both the UV absorbance (254 nm) of the channel outflow as well as absorbance scans (280-700 nm) were measured on the channel outflow. Samples were frozen for EEMS analyses in the shore-based laboratory. In addition to studying spatial variations, vertical profiles also were analyzed, providing an opportunity to examine the differences in the character of CDOM and FDOM upwelling with deep waters compared to that generated by phytoplankton production.

Our progress over the last year has generated a large number of fluorescence excitation and emission spectra (EEMS) measured on samples from different times, places and size fractions. While in some cases (e.g., below) the spectral differences are apparent, a comprehensive intercomparison among these numberous spectra requires a statistical approach. Dr. Jennifer Boehme (Postdoctoral Scientist) has undertaken this task by performing Principle Component Analysis to assess the similarities and differences in fluorescence between both colloidal size fractions and bulk CDOM. Variability within the data set is decomposed into a series of linear terms that can be used to evaluate the relative importance of changes in fluorescence bandwidth and wavelength shifting across size fractions and due to various processes such as dilution and bacterial or photochemical alteration.
The key individuals assisting me with this work are Kathy Hardy (research specialist), Sheri Flogi (M.Sc. student, UM), who is largely responsible for the absorbance measurements, and Jennifer Boehme (Postdoctoral Scientist) who has taken full responsibility for the fluorometric analyses.

RESULTS

1. Flow-FFF provides a distinct and reproducible measure of the size continuum of molecular weight standards added to seawater. Even so, molecular weight standardization provides only a rough estimate of the molecular weights of natural compounds separated by FFFF because molecular shape affects retention times. On-line focusing of 50 ml samples provides enough material for spectral absorbance and fluorescence determinations without biasing the colloidal size distributions.
2. The abundance and size distributions of colloidal CDOM changes significantly between winter and summer in saline waters of the Damariscotta River estuary. Colloidal CDOM is dominated by very small colloids (< ~10 kDa) during winter (low phytoplankton production) and by large colloidal matter (> ~150 kDa) during spring and summer when phytoplankton production is higher. In particular, a large peak at ~ 2 min. retention time appears during the onset of rapid phytolankton growth but diminishes in size as the bloom begins to age. This pattern occurred on two independent blooms. Preliminary assessments of 4F analyese of waters in the Juan de Fuca eddy region are consistent with this small-sized materia being present in regions of high productivity.
3. Laboratory culture experiments demonstrate that marine phytoplankton under some conditions can be a direct source of small and large colloidal CDOM and FDOM, with production rates being highest during the latter stages of culture growth. That is, nutrient-stressed phytoplankton produced larger quantities of colloidal CDOM than rapidly growing cells. Although this finding is in agreement with

earlier experiments with natural population cultures in upwelling waters (see previous annual reports), a recent replicate experiment showed much lower amounts of production, indicating that the growth conditions can significantly affect the release of CDOM.

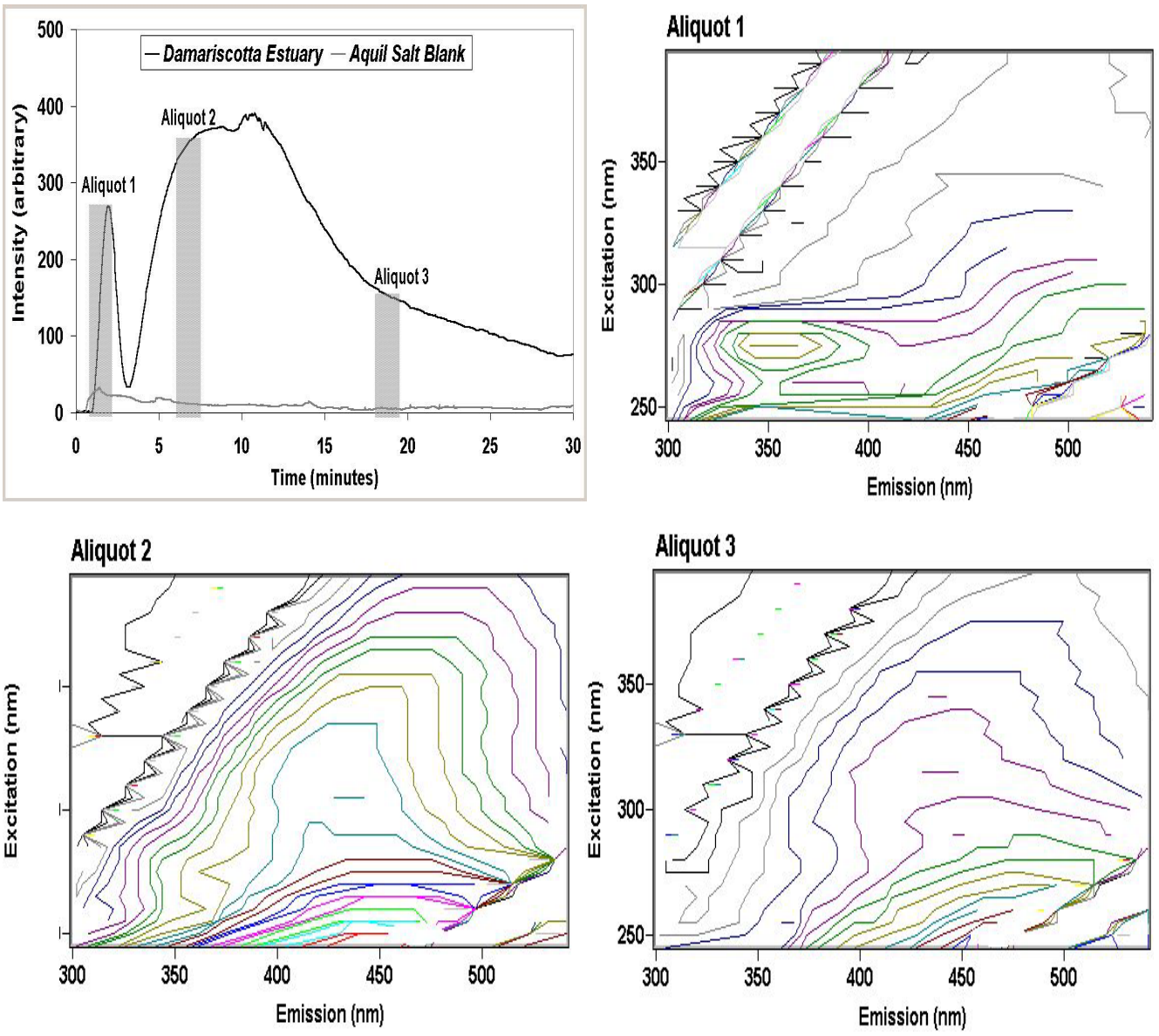

Figure 1. Flow-FFF Fractogram and EEMs of Damariscotta Estuary water, focus volume 50 ml. EEMs display FDOM from selected aliquots collected during sample elution. Collection times are highlighted in gray on the fractogram.

4. The fluorescence characteristics of the colloidal FDOM phase in seawaters from the Damariscotta River estuary show marked differences with size (Fig. 1). Figure 1 depicts the 4F fractogram (254 nM absorbance) as a function of retention time, with three regions being observed; material with a large, short retention time exhibiting "protein-like" dual UV fluorescence at wavelengths similar to tryptophan (Aliquot 1), material within a broader peak displaying dual visible fluorescence peaks generally attributed to humic material (Aliquot 2), and material in a tailing region (Aliquot 3) where the humic fluorescence is red-shifted along emission wavelengths relative to Aliquot 2. These data

provide the best evidence to date that the colloidal FDOM is not homogenous but varies in its optical signature with size. Processes that lead to changes in the colloidal size spectrum in coastal waters thus likely influences the bulk fluorescence signature of seawater.

IMPACT/APPLICATIONS

These findings support the view that a significant fraction of the CDOM and FDOM component in different seawaters are associated with the marine colloidal phase. This colloidal fraction is a dynamic pool that can change over time scales ranging from days to months. The laboratory findings demonstrate that phytoplankton abundance, their growth rates, and the ambient nutrient conditions all will be important factors controlling the in-situ production of colloidal CDOM and FDOM. Marine colloidal matter as a whole is recognized to both aggregate to form large sinking particles, and to serve as a carbon resource for heterotrophic bacteria, both processes of which could influence the bulk CDOM signature of seawater over short time scales. Previous work on this project has demonstrated that the colloidal organic phase harbors hydrophobic microenvironments (based on dye absorption experiments). Partitioning of fluorophores into these reservoirs conceivably could cause fluorescence characteristics of these molecules to shift, a change that might mistakenly be attributed to diagenesis. These findings provide insights to the underlying biogeochemical mechanisms affecting the magnitude and character of CDOM in seawater and complement those studies examining direct photochemical effects.

TRANSITIONS

The application of flow field-flow fractionation methods to the study of the marine organic phase is in its infancy. We anticipate the findings from this work will have wide application among researchers studying CDOM and FDOM in marine and freshwaters.

REFERENCES

Baskaran, M., Santschi, P.H., Benoit, G. and Honeyman, B.D., 1992. Scavenging of Th isotopes by colloids in seawater of the Gulf of Mexico. Geochim. Cosmochim. Acta, 56: 3375-3388.

Benner, R., Pakulski, J.D., McCarthy, M., Hedges, J.I. and Hatcher, P.G., 1992. Bulk chemical characteristics of dissolved organic matter in the ocean. Science, 255: 1561-1564.

Buesseler, K. et al., 1996. An intercomparison of cross-flow filtration techniques for sampling marine colloids: Overview and organic carbon results. Mar. Chem., 51: 1-31.

Chen, Y. and Schnitzer, M., 1989. Sizes and shapes of humic substances by electron microscopy. In: M.H.B. Hayes, P. MacCarthy, R.L. Malcolm and R.S. Smith (Editors), Humic Substances II. John Wiley and Sons, pp. 621-638.

Chin, W.-C., Orellana, M.V. and Verdugo, P., 1998. Spontaneous assembly of marine dissolved organic matter into polymer gels. Nature, 391: 568-572.

Coble, P.G., 1996. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Mar. Chem., 51: 325-346.

Moran, S.B. and Buesseler, K.O., 1992. Short residence times of colloids in the upper ocean estimated from ^{238}U - ^{234}Th disequilibria. Nature, 359: 221-223.

Wells, M.L. and Bruland, K.W., 1998. An improved method for rapid preconcentration and determination of bioactive trace metals in seawater using solid phase extraction and high resolution inductively coupled mass spectrometry. Mar. Chem., 63: 145-153.

PUBLICATIONS

Wells, M. L. 2002. Interactions among marine colloids and metals in seawater: A review. Chapter 11 in "Biogeochemistry of Marine Dissolved Organic Matter", Ed. D.A. Hansell and C. A. Carlson [Published]

Wells, M.L., The colloidal size spectrum of CDOM in the coastal region of the Mississippi Plume using flow field-flow fractionation. ***Marine Chemistry*** [in review].

Chen, R.F. et al., in review. Chromophoric dissolved organic matter (CDOM) source characterization in the Louisiana Bight. ***Marine Chemistry*** [in review].